OPTOELECTRONIC METHODOLOGY SUITABLE FOR ELECTRORETINOGRAPHIC INVESTIGATION OF THE RAT EYE.

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17. **ABSTRACT**
    - A workable optoelectronic arrangement suitable for biomedical investigation in the field of electroretinography (ERG) was devised and is explained. ERGs were obtained in a nontraumatic manner from rhesus monkeys in an altitude chamber and under acceleration. The rhesus monkeys were exposed to a sequence of diffused flashes of light. Silver-silver-chloride electrodes attached to the monkey's head were used to pick up the electroretinal signals caused by the flashes of light. The active electrode was attached to the lower eyelid with the reference electrode at the ipsilateral ear and the ground electrode at the lateral... Cont
canthus. The electroretinal signals were amplified recorded and then analyzed, using an averaging technique. Self-explanatory schematics detail the arrangement and type of instrumentation used. Cerebral oxygen tissue partial pressure (PO2) data, determined by an invasive polarographic method, were collected simultaneously with the ERG to search for a correlation between the two items. Eye safety considerations show that the flashes of light were of an intensity which is considered safe for non-human primates as well as for human subjects for an unlimited experimentation exposure time.
PREFACE

This technique was developed in the Acceleration Effects Branch of the Biodynamics and Bioengineering Division, Air Force Aerospace Medical Research Laboratory (AFAMRL), Wright-Patterson Air Force Base, Ohio. This effort was supported, in part, by the Laboratory Director's Fund 79–8. David Rattino and Abbott Klassen served as project officers for AFAMRL. Radamas Gabel, Alva Karl, and Diana Nelson were project engineers for Systems Research Laboratories, Inc., 2600 Indian Ripple Road, Dayton, Ohio.

The authors acknowledge the assistance of MSgt Gregory D. Bathgate, TSgt Steven D. Bolla, and SSgt Kenneth E. Riggs for their fabrication and setup of the instrumentation, and Mrs. Sandra Noble for help in preparing this report. Photograph by TSgt Anthony Smith.
INTRODUCTION

This report describes the methodology of an electro-optical arrangement suited for collecting electroretinograph (ERG) signals from rhesus monkeys in a nontraumatic fashion. The ERGs were collected under two different environmental conditions. First, the monkeys were placed in the USAF Medical Center's Physiological Training Division altitude chamber. Then the study was rerun with the monkeys placed on the AFAMRL's Dynamic Environment Simulator. The ERG signals were analyzed for correlation with cerebral tissue PO$_2$ data obtained by invasive polarographic methods. We hope the results found by the correlation, which were reported by Kiesen et al. (1980) and Karl et al. (1980) will allow determination of cerebral tissue PO$_2$ by evaluating nontraumatic ERGs during environmental stress. Such stress may include acceleration or high altitude oxygen deprivation. In the past determination of cerebral tissue PO$_2$ has been limited to invasive techniques that restrict its applications.

The ERG method described in this paper may have applications in tasks other than PO$_2$ measurements. All information needed to duplicate the instrumentation is given in detail.
APPRAOH

Significant information concerning the needed opto-electron arrangement (intensity of light, repetition rates, electrode suitability, amplifier characteristics, etc.) was not available when we started this project. At first, we assumed that strong flashes of light (of >400 µjoules/cm² retinal per flash), as supplied by a xenon flash lamp, were needed to obtain useful signals when performing the electroneurography for this project. During preliminary investigations, low intensity light (~0.2 µjoules/cm² retinal per flash) was determined sufficient to produce usable signals.

During the early phase of this task an intolerable number of signal artifacts were caused by muscle movements and to maintain good electrical contact over a prolonged time was practically impossible with some types of electrodes. Initially, commercially available mylar coated electrodes (inserted under the eyelid) were used for the ERG. Strong artifacts were produced by the mylar electrodes and their signal-producing capability deteriorated in about 3 or 4 minutes. Another problem was that the silver-silver-chloride electrodes used for the electrocardiograph (ECG) yielded significant dc potential differences between themselves and the mylar electrodes. Thus silver-silver-chloride electrodes were used for both the ECG and ERG. However, even with the care taken in choosing the type of electrodes, reliable ERG signals could only be obtained by signal averaging (using a Nicolet model No. 1072). The signal-averaging instrument requires sync pulses for correlation with the desired sections of the ERG to be analyzed. Therefore sync pulses, indicating the occurrence of light flashes, were recorded simultaneously with the ERG on a multichannel analog tape recorder.

As stated previously, at the start of this endeavor we assumed that strong flashes of light were needed for obtaining sufficient ERG signal amplitudes, but found that the relationship between light amplitude and ERG amplitude was nonlinear over the range observed. This nonlinear relationship was not investigated further.

In the beginning of the investigation the Super Strobe from Divertronics (Model Luma Power Super Strobe Serial No., 5122) gave erroneous traces because the strobe synchronizes with the 60 Hz power line frequency. Since the 60 Hz are time locked to the ERG stimulus, it appears on the summed potential. If the 60 Hz were random with respect to the stimulus, it would average out. Thus, the strobe approach was abandoned; and a 300-watt Kodak slide projector with an electromechanical Uniblitz shutter were used. Figures 1 and 2 show the detailed final arrangement used for obtaining the electronephelial signals that were correlated with the cerebral PO₂ during baseline and experimental conditions.
Figure 1. PHYSICAL ARRANGEMENT AND LIGHT MEASUREMENTS OF EXPERIMENTAL SETUP
Figure 2. Instrumentation Used To Obtain ERG And PO₂ Signals
INSTRUMENTATION

The instrumentation used in collecting the bioelectric signals during the centrifuge studies is shown in Figures 1 and 2. The altitude chamber studies were conducted with essentially the same equipment. Configuration differences used in the altitude chamber are also discussed.

CENTRIFUGE

The oxygen monitor, TV camera, electroretinogram (ERG) amplifier, electrocardiograph (ECG) amplifier, light source (Kodak Carousel slide projector), shutter, shutter driver, 2-Hertz shutter driver oscillator (Figure 3), and sync pulse generator were all mounted outside the centrifuge cab on the platform shown in Figure 4. Care was taken to insure that all equipment would operate satisfactorily under the stress of high acceleration. The Carousel light source, in the course of the studies, was subjected up to 5 G, for more than 24 runs without any noticeable degradation in its performance.

The signals obtained from this equipment were fed through the centrifuge slip-rings to instrumentation in the monitoring room. The ECG and ERG signals were amplified to at least one volt before being sent through the slip-rings to minimize the effect of slip-ring noise.

ALTITUDE CHAMBER

In the altitude chamber studies, the Carousel projector, shutter, shutter driver, shutter driver oscillator, PO₂ monitor, and sync pulse generator were located inside the chamber with the subject. Instead of the digital readout Critikon Model 4000 INVIVOX™ shown in Figure 2, one with a d'Arsonval movement, called the Critikon Model 145 Multi-Purpose Differential Oxygen Analyzer, was used. The PO₂ analyzer was read through the observation window of the chamber. Both Critikon models are battery operated and therefore isolated from ground to avoid possible shock hazards involving the PO₂ implanted probe.

The ERG and ECG electrode cables were run via tubes through the altitude chamber wall to their respective amplifier located outside the chamber. A sync pulse cable and a cable to provide remote ON/OFF control of the shutter oscillator were also run out of the chamber. The tube space not occupied by the cables was sealed to make the chamber air tight.

The front surface mirror shown in Figure 1 was not used in the altitude chamber. Instead the light went directly from the projector onto the face of the diffusing globe. In both cases the light patch was fixed at 25 inches. The Carousel projector was not used to project slide images, but only as a light source. Figure 1 shows the various photometric and radiometric measurements taken at points in the light patch of the centrifuge configuration.

In both the centrifuge and altitude chamber studies, the ECG, ERG, and sync pulses were recorded on a 7-channel analog tape recorder. The ECG and ERG were recorded with the tape recorder input sensitivity set to 5Vpp while the input sensitivity for the sync pulses was set to 10Vpp. The tape recorder was set at 7/8 from Ips, which provided a 2.5KHz bandwidth.

A closed-circuit video system consisting of the TV camera and monitor was used on the centrifuge to read the PO₂ values and yet keep the oxygen monitor isolated from any ground connections. As previously mentioned, the PO₂ analyzer placed in the altitude chamber was read through the chamber’s window.

The sync pulse generator used in the studies provided the information when the shutter was open. The Carousel light source was kept on during the runs. The 2-Hertz oscillator driving the shutter, located in front of the projector, was turned on and off by means of a remote control switch. The shutter remained open 10 milliseconds for each oscillator pulse it received.
The sync pulse generator used a photo transistor positioned to detect the occurrence of flashes. The pulses obtained from the phototransistor were recorded on tape and later used to sync the Nicolet instrumentation. The Nicolet was used to compute the average transients of the ERG for a series of 64 light flashes as denoted by 64 sync pulses. The Nicolet converted the analog signal to digital, summed the sixty-four 250-millisecond records and displayed the results on a CRT plot of ERG amplitude versus time. An x-y recorder was used to make a permanent copy of the CRT display.

**EYE SAFETY CONSIDERATIONS**

Since a shutter was used to obtain pulsed light from a continuous wave light source, the worst case to be considered for a safety analysis is that the shutter might stay open. If one finds that the light level is safe for even that condition, then it is safe for all shutter operating conditions. Hence, the safety calculations presented here are made for an open shutter, that is, under continuous wave conditions. The calculations show that the configuration satisfies present AFAMRL safety standards.

For cw conditions, the relationship between a pupil dose \( N_p \) [W] for a measured corneal dose \( N_c \) [W/cm\(^2\)] for a given pupil diameter \( d_p \) [cm] is given by

\[
N_p = N_c \times A_p
\]

where \( A_p = \frac{\pi(d_p)^2}{4} \)

Let \( d_p = 0.35 \) cm for a non-human primate. The \( N_c \) radiometric measurement inside the globe was 0.3 \( \times 10^{-3} \) W/cm\(^2\) \( \pm 35\% \). (The \( \pm 35\% \) is because of varying eye position).

Therefore

\[
N_p = 0.3 \times 10^{-3} \text{W/cm}^2 \times \left[ \frac{\pi(0.35 \text{ cm})^2}{4} \right]
\]

\[
N_p = 0.0289 \times 10^{-3} \text{ W} = 28.9 \mu \text{W}
\]

Now one has to determine or to assume over what retinal area \( A_m \) this \( N_p \) is distributed. The following sketches and calculations show how the distribution of \( N_p \) was determined.

For the above \( r \), consider the effective focal length \( (f) \) of monkey's eye to be 12.6 mm. From the well known relationship \( \frac{1}{f} + \frac{1}{b} = \frac{1}{l} \), one finds for \( r = 19 \) cm that \( b = 13.5 \) mm.
The retinal area of the monkey's eye \( A_{m} \) is \( \pi(d/2)^2 \) which equals 2.43 cm\(^2\).

Hence the pupil dose of 28.9 \( \mu \)W was spread over an area of 2.43 cm\(^2\) (eye transmission \( \eta e = 1 \)).

Therefore the retinal density \( N_{m} \) for \( cw \) would be

\[
N_{m} = \frac{Np}{A_{m}} = \frac{28.9 \, \mu W}{2.43 \, \text{cm}^2} \approx 11.9 \, \mu W/\text{cm}^2 \text{ retinal} \pm 35%
\]

which for the \( cw \) value is

\[
\frac{11.9}{40} \approx 0.30 = 30\% \text{ of the Farmers' Standard}
\]

The 40\( \mu \)W/cm\(^2\) value denoted as the "Farmers' Standard" was derived for experiments conducted at AFAMRL with human subjects. Its derivation is explained in Appendix B. It is applied here to the eye safety calculations involving monkeys. Basically, the 40\( \mu \)W/cm\(^2\) is the retinal power value that occurs (assuming eye transmission of \( \eta e = 1 \)) when a farmer is subjected to the sun in a field on a bright day with a ground reflection \( \eta G = 0.1 \).

However, since the \( 11.9 \times 10^{-6} \)W/cm\(^2\) is for \( cw \), meaning \( 11.9 \times 10^{-6} \) J/sec\(^{-1}\) cm\(^{-2}\) and since only two pulses per second with a duration of 10 milliseconds per pulse were used, the effective time \( t_{m} \) is only 20 milliseconds per second. Therefore, the \( 11.9 \times 10^{-6} \) J sec\(^{-1}\) cm\(^{-2}\) energy value has to be corrected for that situation. That is, 20 millisecond per second means only 20/1000 of the time was used. Thus, \( [(11.9 \times 10^{-6} \, \text{J sec}^{-1} \, \text{cm}^{-2})] /50 \pm 35\% \) which is \( 0.238 \times 10^{-6} \, \text{J sec}^{-1} \, \text{cm}^{-2} \pm 35\% \) was actually incident to the retina.

If the high power setting were used on the Carousel projector, the measured light intensities would be increased by approximately 33%.

The previous calculations show that 30% of the Farmers' Standard value is attained under the open shutter condition i.e., shutter failure. Therefore the set up is considered safe.
CONCLUSION

The technology presented in this report proved to be a valuable investigative tool in experimentation carried out in our laboratory using nonhuman primates (Kissen et al., 1980).

For investigations involving human subjects, the volunteer would not need to be placed inside of a diffusing globe (see Figure 1) but could be located behind a ground glass screen. The globe was needed for the monkeys since they may not always be looking straight ahead. The ERG method of this paper is nontraumatic compared with other methods using corneal electrodes.

The eye safety data used in this report are based on the AFAMRL safe exposure standard (40\(\mu\)W/cm\(^2\)) for humans for unlimited exposure time, hence was considered applicable to the rhesus monkey.
APPENDIX A

EQUIPMENT SPECIFICATIONS

Computer Average of Transients

The following instrumentation was used to sum the ERG recordings for analysis.

1. NICOLET Instrument Corporation
   Model NIC-283A
   Magnetic Tape Coupler

2. NICOLET Instrument Corporation
   Model 1072 Instrument Computer
   Settings used: 1024 mag/div (y axis) Vertical Display Scale
                  250 msec/scan (x axis)
   Autostop setting = 64

3. NICOLET Instrument Corporation
   Model SD-72/2A Two Channel Signal Digitizer
   Input A Settings used: filter time constant = 4 milliseconds
                          DC Level and Gain = 4
                          Resolution = 9 bits

4. NICOLET Instrument Corporation
   Model SW71B Wide Range Sweep Control
   Settings used: Dwell time = 244 sec
                  Trigger = +

SRL LOW GAIN BIOPOTENTIAL AMPLIFIER

The module meets the following requirements:
  Responses: 0.05 to 5000 Hz
  Input: differential
  Input Impedance: > 2 megohm
  Common Mode Rejection: 90 db at 60 Hz not damaging
  Input Noise: < 3 microvolts rms (shorted input)
  Gain: adjustable in 4 steps
        1000, 2000, 5000, 10000
  Output: ± 10 volt

SRL HIGH GAIN BIOPOTENTIAL AMPLIFIER

The high gain biopotential amplifier specifications are identical to the specifications for the low gain biopotential amplifier, with a few exceptions.
  Response: 0.5 to 5000 Hz
  Gain: adjustable in 4 steps
        10000, 20000, 50000, 100000
APPENDIX B

"FARMERS STANDARD"

The following is a brief synopsis of the "Farmers' Standard" which was conceived by co-author R.K.H. Gebel.

The "Farmers' Standard" is based on the following facts or assertions:

1. The incident power of the sun's radiation \( N_a \) at noon, on a cloudless day, at sea level, on the ground is approximately equal to 115 mW/cm\(^2\). This value of \( N_a \) is for a \( \lambda \) from 280 nm to 1.45 \( \mu \)m as spectrally tabulated by Gebel et al., "An Introduction to the Problem of Photographing Artificial Satellites", the Ohio Journal of Science 62(4) 191, July 1962.

2. The average reflection of the ground \( \eta_d(\lambda) \), as ordinarily experienced by a farmer, is assumed to be 10 percent for white light.

3. The transmission efficiency of the human eye \( \eta_v(\lambda) \) for white is about 50 percent.

4. The effective focal length of the human eye \( f_e \) is approximately equal to 17 millimeters.

5. The effective pupil diameter \( d_p \) of the human eye (variable from 2 millimeters to 7 millimeters) is about 2 millimeters for bright daylight.

The relationship of these five parameters to the retinal power incidence \( N_r \) is expressed by the equation

\[
N_r = \frac{N_a \times \eta_d(\lambda) \eta_v(\lambda)}{4\left(f_e/d_p\right)^2} \left[ \frac{W}{cm^2} \right]
\]

\[
N_r = \frac{(115)(.1)(.5)}{4\left(17/2\right)^2} = \frac{5.75}{289} \approx 20 \mu W/cm^2
\]

By neglecting the transmission efficiency of the eye, the power incident on the retina is 40 \( \mu \)W/cm\(^2\). This 40 \( \mu \)W/cm\(^2\) value is denoted as the "Farmers' Standard".
REFERENCES
